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TITLE: Pathogenesis and Prediction of Rheumatoid Arthritis

PRINCIPAL INVESTIGATOR: Kevin D. Deane, MD/PhD

CONTRACTING ORGANIZATION: University of Colorado Denver
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13. SUPPLEMENTARY NOTES

14. ABSTRACT

It is now well established that there is a preclinical period of rheumatoid arthritis (RA) development that is characterized by abnormalities of the immune system prior to the onset of the clinically apparent inflammatory joint disease that currently defines RA. The primary goal of this project is to investigate this preclinical period in order to understand two major factors: 1) how biomarker changes in preclinical RA can be used to accurately predict the future development of RA in currently asymptomatic individuals, and 2) to identify factors related to the pathogenesis of RA that can ultimately be targeted to prevent RA. This project has proposed to use a unique set of serum samples and clinical data available through the Department of Defense Serum Repository (DoDSR) to investigate the preclinical period of RA. During the first two years of this project (30 Sep 2013-29 Sep 2015) we have acquired the serums samples and data from the DoDSR, and performed biomarker testing. Final analyses of these data will be performed in Year 3 (2015-2016).

15. SUBJECT TERMS

Prediction of future rheumatoid arthritis; pathogenesis of rheumatoid arthritis

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INTRODUCTION:
This is the second Annual Report for the project entitled "Pathogenesis and Prediction of Rheumatoid Arthritis", PI Kevin D. Deane, period 30 Sep 2014 – 29 Sep 2015. The date that this report is submitted is 29 Sep 2015.

It is now well established that there is a preclinical period of rheumatoid arthritis (RA) development that is characterized by abnormalities of the immune system prior to the onset of the clinically apparent inflammatory joint disease that currently defines RA. The primary goal of this project is to investigate this preclinical period in order to understand two major factors: 1) how biomarker changes in preclinical RA can be used to accurately predict the future development of RA in currently asymptomatic individuals, and 2) to identify factors related to the pathogenesis of RA that can ultimately be targeted to prevent RA.

This project has proposed to use a unique set of serum samples and clinical data available through the Department of Defense Serum Repository (DoDSR) to investigate the preclinical period of RA.

As described below in more detail, during the first year of this project, we have acquired the serums samples and data from the DoDSR, and performed initial biomarker testing.

KEYWORDS:
Pathogenesis of rheumatoid arthritis
Prediction of rheumatoid arthritis

ACCOMPLISHMENTS:
What were the major goals of the project?
For the first two years of the project, as stated in the Statement of Work (SOW) the major goals/tasks were as follows:

1) Clinical data and sample procurement from the DoDSR. (SOW Task 1)
2) Obtain regulatory IRB/HRPO approval. (SOW Task 2)
3) Research assistant hiring and training. (SOW Task 3)
4) RA-related autoantibody testing in 1600 serum samples from 200 RA cases and 200 healthy controls. The specific autoantibodies include testing for anti-cyclic citrullined peptide (anti-CCP)-2, anti-CCP3.1, rheumatoid factor isotypes, and an array for antibodies to citrullinated protein antigens (ACPAs). (SOW Task 4)
5) Testing antibodies to oral pathogens in 1600 serum samples from 200 RA cases and 200 healthy controls. (SOW Task 6).
6) Testing serum samples for cotinine levels (SOW Task 7).

What was accomplished under these goals?
Task 1. We obtained clinical data and serum samples from the DoDSR. These samples are now housed at the University of Colorado Denver in Dr. Deane's lab and the serum

samples have used to perform the tests as listed below. Clinical data was obtained per medical chart review and includes the items listed in the original SOW as follows: subject age, gender, race, region of enlistment and military specialty, time of onset of RA and symptoms, classification criteria met for RA, medication use pre and post-RA diagnosis. In addition, other medical illnesses and other environmental exposures such as smoking, periodontal disease were ascertained.

Task 2. Local and governmental IRB approvals, and HRPO approval, were obtained for this project.

Task 3. Research assistants were hired and trained for this project in the first year of this project and continued their work in the second year. Please see the 'Participation' section below for details of these individuals.

Task 4. We completed serum sample testing for each of the following:
- Anti-CCP2 using ELISA kits (Axis-Shield) and established methodologies.
- Anti-CCP3.1 using ELISA kits (INOVA) and established methodologies.
- Rheumatoid factor isotypes (A, G and M) using ELISA kits (INOVA) and established methodologies.
- ACPA arrays

To date to preserve the scientific integrity of the project, we are remaining blinded to subject status (e.g. RA case v. control), and full un-blinded analyses of these results are planned for year 3 of this project. However, as reported in last year's Annual Report (and in Table 1 below) there are numerous samples that are positive for RA-related autoantibodies supporting that we will have sufficient outcomes for robust analyses.

Table 1. Preliminary Results of Autoantibody Testing of 1600 Samples and Blinded to RA Case v. Control Status

Autoantibody Test	% positive (of 1600 samples)
CCP3.1	497 (31%)
IgA-RF	235 (15%)
IgM-RF	487 (30%)
IgG-RF	448 (28%)
Any Ab	608 (38%)

Task 6. We have completed testing for oral pathogens *Porphyromonas gingivalis, Prevotella intermedia* and *Fusibacterium nucleatum.* This was performed in the lab of Drs Ted Mikuls and Geoffrey Thiele using established protocols. Full analyses of these results are planned for year 3 of this project.

Task 7. We are in the process of completing cotinine analyses on the 1600 samples. This should be completed by December 2015.

What opportunities for training and professional development has the project provided? In this initial phase of the project(years 1-2), activities did not include training and professional development. However, in year 3 of the project we expect that this project will allow for early career rheumatologists including fellows and junior faculty to participate in scientific research. During the next year of the project, these activities will expand through more detailed analyses and publications, and additional personnel will be posted when they become known.

How were the results disseminated to communities of interest? Nothing to report because during the first 2 years of the three-year project, our goals were to perform data and sample acquisition, and initial sample testing.

What do you plan to do during the next reporting period to accomplish the goals? During the next and final year of the project, we will complete sample testing, and then perform analyses as described in our Statement of Work.

IMPACT:

What was the impact on the development of the principal discipline(s) of the project? Nothing to report at this phase of the project.

What was the impact on other disciplines? Nothing to report at this phase of the project.

What was the impact on technology transfer? Nothing to report at this phase of the project.

What was the impact on society beyond science and technology? Nothing to report at this phase of the project.

CHANGES/PROBLEMS:

Changes in approach and reasons for change There have been no substantive changes in the overall approach. However, we had initially allocated a certain amount of funds to obtain the serum samples from the DoDSR; however, we needed fewer funds than anticipated. We petitioned the DoD to use these remaining funds for additional testing of the serum samples; specifically to test for isotypes immunoglobulin (Ig) A, IgG and IgM to citrullinated proteins. We obtained approval to complete this testing, and we have completed this testing. Please see revised SOW at the end of the Annual Report for additional details.

Actual or anticipated problems or delays and actions or plans to resolve them Due to funding cut-backs at the DoD level as well as military-based issues with personnel who were able to procure clinical data from the DoD, we have had delays in obtaining the clinical data from our subjects. However, we have surmounted these issues and will have

the full clinical data linked to the biomarker testing available by October 2015, allowing for completion of analyses as planned. Also, we had delays in obtaining cotinine ELISA kits but now have those in hand and will complete that testing by December 2015.

Changes that had a significant impact on expenditures Please see 'Changes in Approach' above.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents Nothing to report.

Significant changes in use or care of human subjects Nothing to report.

Significant changes in use or care of vertebrate animals Nothing to report.

Significant changes in use of biohazards and/or select agents Nothing to report.

PRODUCTS:

Publications, conference papers, and presentations All analyses and resultant publications are planned for year 3 of this project; as such at this time, there is nothing to report.

Journal publications Nothing to report.

Books or other non-periodical, one-time publications Nothing to report.

Other publications, conference papers, and presentations Nothing to report.

Website(s) or other Internet site(s) Nothing to report.

Technologies or techniques Nothing to report.

Inventions, patent applications, and/or licenses Nothing to report.

Other Products Nothing to report.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project? There are no changes related to the original investigators on the project as reported in the original proposal with the exception that research assistants have been named for the project. The investigators and research assistants are as follows (and listed in alphabetical order after the PI).

Name	**Kevin D. Deane, MD/PhD**
Project Role	PI
Nearest Person Month Worked	2
Contribution	Oversee the entire project
Funding Support	This project

Name	**Jess Edison, MD**
Project Role	Co-investigator
Nearest Person Month Worked	1
Contribution	Data extraction; sample management at DoD
Funding Support	Dr. Edison is paid through his position as active duty military and receives no funds from this project.

Name	**V. Michael Holers, MD**
Project Role	Co-investigator
Nearest Person Month Worked	1
Contribution	Oversee biomarker testing
Funding Support	This project

Name	**Ted R. Mikuls, MD/MSPH**
Project Role	Co-investigator
Nearest Person Month Worked	1
Contribution	Testing for antibodies to oral pathogens
Funding Support	This project

Name	**William Robinson, MD/PhD**
Project Role	Co-investigator
Nearest Person Month Worked	1
Contribution	Testing ACPA array
Funding Support	This project

Name	**Jeremy Sokolove, MD**
Project Role	Co-investigator
Nearest Person Month Worked	1
Contribution	Testing ACPA array
Funding Support	This project

Name	**Geoff Thiele, PhD**
Project Role	Co-investigator
Nearest Person Month Worked	1
Contribution	Testing for antibodies to oral pathogens
Funding Support	This project

Name	**Gary O. Zerbe, PhD**
Project Role	Co-investigator
Nearest Person Month Worked	1
Contribution	Statistical analyses; study design and power
Funding Support	This project

Name	**Marie Feser, MSPH**
Project Role	Study Coordinator
Nearest Person Month Worked	12
Contribution	Oversee the entire project
Funding Support	This project

Name	**Mark Parish, BA**
Project Role	Research assistant/laboratory technician
Nearest Person Month Worked	12
Contribution	Laboratory testing/sample management
Funding Support	This project

Name	**Emily Stein, PhD**
Project Role	Research assistant
Nearest Person Month Worked	3
Contribution	ACPA testing
Funding Support	This project

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period? Please see the following table.

Changes in Active Other Support for PI/Key Personnel During the Reporting Period 30 Sept 2014 – 29 Sept 2015	
Kevin Deane, MD/PhD	Dr. Deane has received an appointment at the Denver Veterans Affairs Hospital and received new NIH grant funding; these changes do not affect his effort on this project.
Jess Edison, MD	No changes.
V. Michael Holers, MD	Dr. Holers has received new NIH grant funding and had one grant expire; these changes do not affect his effort on this project.
Ted Mikuls, MD/MSPH	No changes.
William Robinson, MD/PhD	Dr. Robinson has received new NIH funding; these changes do not affect his effort on this project.
Jeremy Sokolove, MD	Dr. Sokolove has received new NIH funding; these changes do not affect his effort on this project.
Geoffrey Thiele, PhD	No changes.
Gary O. Zerbe, PhD	No changes.

What other organizations were involved as partners? There have been no changes from the original proposal in the organizations involved in this project. The organizations that have participated in this project are as follows:

University of Colorado Denver
1775 Aurora Court
Aurora, Colorado USA
The PI Dr. Deane and co-investigator Dr. Holers are based at this institution; all data and samples are housed at this institution.

University of Nebraska Medical Center
986270 Nebraska Medical Center
Omaha, NE 68198 USA
Co-investigators Drs Mikuls and Thiele are based at the University of Nebraska and are performing the testing for antibodies to oral pathogens as well as contributing to the overall design and implementation of the project.

Veterans Affairs Palo Alto Health Care System
3801 Miranda Avenue
Palo Alto, CA 94304
Co-investigators Drs Robinson and Sokolove are based at the Palo Alto VA and are performing testing for the ACPA array and related analyses.

Walter Reed National Military Medical Center
8901 Wisconsin Avenue
Bethesda, MD 20889
Co-investigator Dr. Edison is based at Walter Reed and is obtaining the clinical data and military IRB approvals related to this project.

SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

Not applicable.

QUAD CHARTS:

Not applicable.

APPENDICES

Revised Statement of Work to include additional testing for CCP isotypes.

STATEMENT OF WORK for PR120839 PI: Deane, Kevin

Revised on Oct 20, 2014 with revised portions in yellow highlights

Project Title: PR120839 "Pathogenesis and Prediction of Future Rheumatoid Arthritis"
Principal Investigator: Kevin Dale Deane, MD/PhD

I. INTRODUCTION AND PROJECT OVERVIEW

This revised SOW is submitted based on the request of the Department of Defense (DoD)/Congressional Directed Medical Research Program (CDMRP) regarding PR120839 entitled "Pathogenesis and Prediction of Future Rheumatoid Arthritis", PI Kevin Deane, MD/PhD.

This project will utilize the unique resources of the Department of Defense Serum Repository (DoDSR) to investigate the preclinical period of rheumatoid arthritis (RA) development in order to gain a deeper understanding of the pathogenesis of RA, as well as develop a predictive model for the future onset of RA in currently asymptomatic individuals.

As described in our original grant submission, this is a large project that will evaluate clinical data and test sera samples from an estimated 400 cases with RA and 1600 controls for factors related to RA. Due to the scope of this project, as described in the original application, funding from multiple sources will be used to complete all research-related activities. These funding sources include the DoD, the American College of Rheumatology Rheumatology Research Foundation (ACR-RRF), and the Walter S. and Lucienne Driskill Foundation.

In order to accommodate funding from these sources and avoid significant overlap, we will utilize funding from these sources to perform different aspects of the study. As such, herein, we will describe the research activities that will be funded by the DoD, and include a description of activities that will be funded by the other sources. We will also discuss the relevant research-related activities that will take place prior to the start of DoD funding.

II. DURATION OF PROJECT

Estimated DoD/CDMRP Funding Start Date: 30 Sep 2013
Estimated DoD/CDMRP Funding End Date: 29 Sep 2016

III. TASKS

Task 1. Clinical Data Collection and sample procurement. A total of 8000 serum samples from 400 RA cases and 1600 matched controls will be identified in the DoDSR and samples shipped to the primary study site at the University of Colorado. Clinical data related to the cases and controls will be collected by staff at Walter Reed National Military Medical Center (WRNMMC) under the direction of Jess Edison, and include the items listed in Table 1. Please see Section VII for a complete listing of the institutions and investigators involved in performance of these Tasks.

Table 1. Data collected on military RA cases and controls
RA cases Subject characteristics including: age at time of diagnosis of RA, gender, race, region of enlistment, and military specialty, Time of onset of arthritis symptoms prior to diagnosis of RA; number and type of classification criteria for RA fulfilled based on 1987 classification criteria; presence of erosions, medication use at time of sample collection (including RA medications, and medications that may impact autoimmunity such as hormones) Smoking status (ever vs. never, and pack-years); periodontal disease; alcohol intake and other medical illnesses. **Controls** Same non-RA variables as RA cases, although controls' age will be matched to the date of cases' time of diagnosis of RA.

Performance period: Months 0-3 of the project period.

Methods: Electronic medical record/chart review and sample procurement from the DoDSR

Outcomes/Deliverables: Complete sample and clinical dataset for cases and controls.

Task #2: Obtain regulatory IRB/HRPO approvals. The final DoD CDMRP Human Resources Protection Office (HRPO) approval will be obtained during months 0-3 of this project. Dr. Deane will oversee this process, aided by the Study Coordinator.

Performance period: Months 0-3 of the project period.

Methods: Standard forms and procedures for IRB approvals.

Outcomes/Deliverables: HRPO approval.

Task #3: Research assistant hiring and training. The Study Coordinator and research assistant for this project will be hired as and work with Dr. Deane in completing final IRB approvals for this project, communication with WRNMMC and the DoDSR regarding clinical data and biologic sample collection, development of the study database, and when applicable, training in human subjects research, laboratory techniques and sample management necessary for the project.

Performance period: Months 0-3 of the project period.

Methods: Training of research assistants in human subjects research, laboratory methods; development of the study database.

Outcomes/Deliverables: Fully-trained research assistants and fully developed database.

IVa. Specific Aim #1) To characterize the evolution of biomarkers from a non-autoimmune/non-inflammatory state to clinically-apparent RA *Approach:* For this entire project, 8000 sera samples from before and after the onset of clinically-apparent RA from 400 military subjects with RA and 1600 matched controls will be tested for Abs and other biomarkers using cutting-edge methodologies to evaluate immunologic responses through the transitions from a non-autoimmune/non-inflammatory state to clinically-apparent RA. The specific tests and number of samples analyzed with DoD funding are listed below in Table 2, and represent testing of 200 RA cases and 200 matched controls, each with 4 sera samples available, for a total of 1600 samples.

Table 2. Biomarker Testing					
	Location of testing	**Total samples tested with DoD/CDMRP funding[1]**	**Year 1**	**Year 2**	**Year 3**
Autoantibody Testing					
- Anti-CCP2	CU	1600	1600	-	-
- Anti-CCP3.1	CU	1600	1600	-	-
- RF isotypes	CU	1600	1600	-	-
- ACPA array	Stanford	1600	1600		
Antibody to oral pathogens	UNMC	1600	1600	-	-
Cotinine levels	CU	1600	-	1600	-

Task 4. RA-related autoantibody testing. Please see Table 2 for a listing of the autoantibodies to be tested for this project. Testing for anti-CCP2, anti-CCP3.1 and RF isotypes will be performed at the University of Colorado (CU) under the direction of Kevin Deane and Michael Holers. The ACPA array will be performed at Stanford University (SU) under the direction of William Robinson and Jeremy Sokolove.

Performance period: Months 4-12 of the project period. Please see Table 2 for the specific testing that will be performed during each year of the project.

Methods: CCP and RF testing will be performed using commercial ELISA assays. ACPA array testing will be performed using bead-based array technology established by William Robinson.

Outcomes/Deliverables: Numeric values for each test that can be used in continuous or dichotomous fashion for analyses.

Task 5. Analyses for Specific Aim 1. These analyses include a) determining diagnostic accuracy of RA-related biomarkers, 2) determining the timing of appearance of biomarkers relative to each other in the preclinical period of RA, and 3) evaluating the evolution of particular biomarkers over time in preclinical RA.

Performance period: Months 25-36 of the project period.

Methods: The analytic methods to be used for Task 5 are as follows.

Diagnostic accuracy of biomarkers for RA: We will evaluate the diagnostic accuracy (sensitivity, specificity, positive and negative predictive values, and likelihood ratios) of single or combinations/patterns of biomarkers for future RA in a case/control fashion (case: control 1:2) using several approaches including 2x2 table analyses for single markers, receiver operator curve (ROC) analyses for 'counts' of markers (e.g. 0, 1, 2, elevated cytokines/chemokines), and cluster analyses using Significance Analysis of Microarrays [SAM] for combinations of markers. Given the large number of biomarker comparisons, we will also account for multiple comparisons using approaches such as Bonferroni method for individual tests, and false discovery rates for cluster analyses. Using these approaches, we will be able to establish the diagnostic accuracy for each marker in pooled samples (e.g. sensitivity and specificity for a marker in any preclinical sample), and by defined time intervals prior to diagnosis (e.g. sensitivity and specificity of a marker for future RA in the interval of 0-1 years prior to diagnosis). Furthermore, we will also evaluate the diagnostic accuracy of continuous levels of biomarkers, or interval increases, for RA using regression analyses – e.g. higher levels of certain biomarkers may have greater diagnostic accuracy for disease than lower levels.

Evaluating times of appearance of biomarkers in preclinical RA: We will use proportional hazards models with random subject effects (frailty model) that can account for covariates to evaluate the times of appearance of each biomarker during the preclinical period of RA development. This approach will also enable us to estimate median (or other percentile) time to first appearance of each test, and compare medians with a Wald test to determine which biomarker has appeared 'first' in the evolution of RA. We have already utilized these methods in our prior DoDSR sample set analyses, and using this

experience in these new analyses we will using the time of diagnosis of RA as the start point, and work backwards in time, with the appearance of a biomarker abnormality (as dichotomous variable) as the event. This approach will also include interval and left censorship to account for subjects whose earliest samples had a biomarker abnormality.

Evolution of biomarkers We will evaluate the evolution of biomarkers during RA development using mixed model regression analyses that can account for subject effects as well as covariates. The outcome variable for these analyses will be RA versus no RA to compare the evolution of these factors in RA to controls, and predictor variables can be continuous or dichotomous. Additionally, in mixed model regression analyses, we will evaluate evolution of these factors within RA cases prior to and after their diagnosis of RA to determine the relationship between these factors and development of clinically-apparent RA, with the outcome variable being preclinical status versus clinically-apparent RA.

Outcomes/Deliverables. We expect that, when assessed in the preclinical period of RA development, patterns and phenotypes of biomarkers are highly predictive of future RA. We also expect that certain biomarkers precede others, and that the sequence of appearance these biomarkers, and alterations of pathogenicty of Abs over time, are indicative of a progression of biological pathways that are important in the pathogenesis of RA. For example, results from ACPA array testing may identify the earliest autoimmune responses to specific citrullinated proteins; thereby informing us with the proteins that are part of the initial break in immune tolerance in RA, and that in future work may be targets to induce immune tolerance for disease prevention.

Outcomes/Deliverables: Completed analyses.

IVb. Specific Aim #2) To examine the relationship between subject characteristics and specific environmental exposures that have been identified as risk factors for RA, and incident and evolving RA-related autoimmunity and inflammation prior to the onset of clinically-apparent RA. *Approach:* We will evaluate the relationship between subject characteristics and historical and serologic evidence of exposure to tobacco smoke and infection with pathogens in relationship to the incidence and evolution of autoimmune and inflammatory biomarkers and the development of clinically-apparent RA.

Task 6. Antibody to oral pathogens. Testing for antibody to the following oral pathogens will performed at the University of Nebraska Medical Center (UNMC) under the direction of Drs Ted Mikuls and Geoff Thiele: *Porphyromonal gingivals, Prevotella intermedia* and *Fusibacterium nucleatum*.

Performance period: Months 4-12 of the project period.

Methods: Antibody to oral pathogens will be tested using ELISA-based assays to bacterial wall outer membranes that have been developed and validated by Drs Mikuls and Thiele.

Outcomes/Deliverables: Continuous and dichotomous values for each test that can be used in analyses.

Task 7. Cotinine testing. Serum testing for cotinine levels will be performed at CU under the direction of Kevin Deane and Michael Holers.

Performance period: Months 13-24 of the project period.

Methods: Cotinine levels will be performed using ELISA-based assay.

Outcomes/Deliverables: Continuous and dichotomous values that can be used in analyses.

Task 8. Analyses for Specific Aim 2. These analyses include determining the temporal relationship between clinical and biomarker evidence of exposure to a variety of factors (tobacco smoke, oral pathogens, etc) and development and progression of RA-related autoimmunity and inflammation, and diagnosis of RA.

Performance period: Months 25-36 of the project period.

Methods: The analyses for Specific Aim 2/Task 7 will be performed through the following methods:

Association of risk factors with RA We will evaluate the relationship between history of exposure to tobacco smoke and periodontal factors with a clinical diagnosis of RA using mixed model regression analyses including cases and controls, that can account for between and within subject effects as well as interaction terms, and determine which measure of exposure (e.g. self-report vs. biomarker) is most predictive of RA. The primary outcome will be RA, and predictor variables will include smoking history, periodontal disease history, and biomarkers of tobacco exposure and infection with oral pathogens, and other covariates as appropriate. Predictor variables can be evaluated as dichotomous, or continuous to determine 'dose' effect.

Timing of exposure and incident RA-related autoimmunity, and RA We will evaluate the temporal relationship between exposures to environmental factors and the appearance of RA-related Abs prior to a diagnosis of RA (e.g. does elevation of antibody to *P ging* precede the appearance of ACPAs?). For these analyses, for dichotomous variables, we will use proportional hazards models described in Aim 1. For continuous variables, we will use mixed models that include a time lag parameter described in Aim 1. Furthermore, we will also use these techniques to predict the incidence of initial RA-related autoimmunity. For example, certain factors such as exposure to tobacco smoke, or elevations of antibody to oral pathogens, many precede the appearance of RA-specific Abs, or may be temporally related to changes in ACPA pathogenicity.

Outcomes/Deliverables: We expect that tobacco smoke, periodontal disease and infection with *P ging* (and perhaps other pathogens) as well as potentially other factors will be strongly associated with risk for future RA and incidence and/or progression of autoimmunity, thereby improving our understanding of the mechanisms of disease development, prediction of future disease, and potentially serving as targets for disease prevention.

IVc. Specific Aim #3) To utilize subject characteristics, biomarkers and environmental exposures to develop the most highly predictive model to predict both the likelihood and timing of future RA. *Approach:* We will utilize biomarkers of autoimmunity, inflammation, and environmental exposures and develop a robust model designed to predict the likelihood and timing of onset of future clinically apparent RA.

Task 9. Predictive modeling for likelihood and timing of future RA. For this task, we will utilize the clinical and biomarker data as described above to develop a predictive model to predict both the likelihood and timing of future RA.

Performance period: This Task requires complete testing and therefore will be completed during months 25-36 of the project.

Methods: In prior work, we developed a 2-step model to predict the likelihood and timing of future clinically-apparent RA. This approach offered best-fit for the data, and furthermore is a clinically-practical approach in real-world settings where one would likely first assess someone's overall risk for RA using highly RA-specific markers, then determine how soon they may develop symptomatic disease. We suspect that we will develop a similar 2-step model in this new project, and our analytic approach will be to initially evaluate the diagnostic accuracy of each clinical factor (age, sex, race, etc) and biomarker that was associated with RA and then determine the factors that most accurately predict the likelihood of RA. We will then use these factors in mixed model analyses to develop a model to predict the timing of future onset of RA with the dependent variable being the time from sample to time to diagnosis that can be a continuous variable (e.g. day to diagnosis) or dichotomous (e.g. <1 year or ≥ 1 year), and predictor variables included in the final model based on a likelihood ratio test. Factors that we believe *a priori* are related to the development of RA include exposure to tobacco smoke, periodontal disease and infection with specific oral pathogens, and elevations of RA-related Abs, and

inflammatory markers. Furthermore, based on our prior work and other published data, the factors we believe *a priori* are related to the *timing* of development of RA include age, tobacco smoke, levels of Abs and inflammatory biomarkers (e.g. higher levels = more imminent disease) and changes in ACPA pathogenicity (e.g. altered G0 content, increased capability of ACPAs to activate complement). These factors will the foci of our analyses; however, we will also explore the relationship between other variables and outcomes.

Outcomes/Deliverables: We expect to develop a model to predict the likelihood and timing of future onset of RA, but importantly a more robust model that those previously developed because of the increased sample size in these experiments. Additionally, this new model will include additional clinical and biomarker factors than were used in our first military RA experiments, further contributing to the robustness and accuracy of this new model. Going forward, this model can be applied in additional populations to identify subjects that are at-risk for future RA in whom preventive measures for RA may be instituted.

V. ADDITIONAL TASKS

Task 10. Interim analyses and potential abstract preparation/submission. At the end of the first and second years of the study we will perform interim analyses of the data for quality control and validation measures. In addition, we will utilize these data to develop interim abstracts regarding our findings. If these findings support submission, we will aim to submit them to the American College of Rheumatology Annual Meeting that has an abstract deadline of late June every year.

Performance period: Months 12-13 and 24-25 of the project period.

Outcomes/deliverables: Completed interim analyses; abstract(s) completion.

Task 11. Final manuscript(s) preparation. At the completion of all testing, we will develop final manuscripts related to our findings.

Performance period: Months 31-36 of the project period.

Outcomes/deliverables: Submitted manuscript(s).

Task 12. Additional studies: Isotype testing to CCP (added 1-Oct-2014). Based on pilot data from other studies as well as emerging published data, we believe that evaluating the evolution of specific isotypes (e.g. IgA, IgG and IgM) to CCP will be informative in preclinical RA. Specifically, in pilot work using a separate cohort of subjects from this DoD sample set, we have found that IgA-CCP elevations appear earliest in the preclinical period of RA development; in addition, we have found that transition to IgG-CCP is a marker of imminent onset of RA (unpublished data). We will therefore test specific isotypes IgA, IgG and IgM to CCP during months 13-24 of the project period.

VI. TIMELINE

Table 3. Project Timeline						
	Planned Task Completion					
Tasks	Year 1		Year 2		Year 3	
	0-3 months	4-12 months	13-18 months	19-24 months	25-30 months	31-36 months
Task 1. Clinical data collection and sample acquisition	X					
Task 2. Regulatory approvals	X					
Task 3. Research assistant hiring and training	X					
Task 4. RA-related autoantibody testing		X				
Task 5. Analyses for Specific Aim 1					X	
Task 6. Antibody to oral pathogens		X				
Task 7. Cotinine testing				X		
Task 8. Analyses for Specific Aim 2					X	
Task 9. Predictive modeling for likelihood and timing of future RA					X	
Task 10. Interim analyses and abstract preparation		X		X		
Task 11. Final manuscript(s) preparation						X
Task 12. *Additional studies: Isotype testing to CCP (added 1-Oct-2014).*			X	X		

VII. INVESTIGATORS

The following are the investigators (in alphabetical order after the Principal investigator) and institutions that will be participating in the above-mentioned Tasks for this project.

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